AD-A036 300 SCHOOL OF AVIATION MEDICINE RANDOLPH AFB TEX F/G 6/16
SEASONAL VARIATION IN HUMAN AMINO ACID EXCRETION,(U)
JUN 59 H B HALE, J P ELLIS, D D VAN FOSSAN.
UNCLASSIFIED SAM-59-80 NL

1 OF 1
AD-A
036 300

END
DATE
FILMED
4-4-77
NTIS

SEASONAL VARIATION IN HUMAN AMINO ACID EXCRETION

SCHOOL OF AVIATION MEDICINE
RANDOLPH AIR FORCE BASE, TEXAS

JUNE 1959

SEASONAL VARIATION IN HUMAN AMINO ACID EXCRETION

HENRY B. HALE, Ph.D.
JAMES P. ELLIS, Jr., M.A.
DONALD D. VAN FOSSAN, Ph.D.

Department of Physiology and Biophysics

59-80

Air University
SCHOOL OF AVIATION MEDICINE, USAF
RANDOLPH AFB, TEXAS
June 1959

7758-82

SEASONAL VARIATION IN HUMAN AMINO ACID EXCRETION

Amino acid excretion was studied in young, healthy men during summer, fall, and winter months in a location in southwestern United States. Both random and timed urine samples were employed. The amino acids determined were alanine, arginine, cysteine, glutamic acid, glutamine, glycine, histidine, lysine, methyl histidine, serine, threonine, and valine. Supplemental determinations included urine volume, creatinine, uric acid, urea, sodium, and potassium. Using random samples, and expressing values as ratios with creatinine, significant seasonal variation was found for alanine, arginine, cysteine, glutamic acid, glycine, lysine, serine, urea, and uric acid. Although there was no significant variation in urine volume, both creatinine excretion and the urinary Na/K ratio varied significantly with season. These effects are interpreted as the result of changes in glomerular filtration and tubular reabsorption rates.

Seasonal variation in urinary amino acids was noted by Mefferd et. al. (1) among hospitalized schizophrenics. The question was raised whether healthy, active persons also show such variation, since in patients, the factors of disease and inactivity may have contributed greatly to the changes noted. In laboratory animals, urinary excretion of certain amino acids tends to be temperature-dependent (2-4). To attempt to answer this question, groups of healthy male subjects were studied in summer, fall, and winter months in a geographic location (southern Texas) in which the summer is hot and dry, with peak temperatures regularly approaching 100° F. In the fall and late winter, the weather tends to be mild, and lightweight clothing suffices. The comparison is, therefore, essentially that of hot vs. neutral conditions.

One feature of the study is the use of untimed urine samples. The purpose was to determine whether such samples could be employed in field studies.

METHODS

Young men in military service (laboratory or altitude chamber technicians) served as subjects. Their diet and living conditions were reasonably constant, since all belonged to the same squadron. Untimed urine samples were obtained at the end of the noon hour (1300 hours), but this was the limit of the standardization. The summer specimens (taken in June, July, and August) were obtained from 34 different subjects; the fall (October and November) specimens were taken from 19 additional men; and the winter specimens (February and March) were taken from 30 others.

Timed urine samples were taken subsequently from representatives of each seasonal group. It was not possible to obtain such samples from the others, because they participated in altitude tests. The timed samples were standardized with respect to time of day (1300 to 1500 hours), state of activity (seated), and environmental conditions (the laboratory temperature was controlled on all occasions).

Amino acid determinations were made on desalted urine extracts (5,6) using the ascending two-dimensional paper chromatographic technic. Buffered phenol (7) served as the first solvent, whereas a 4:1:5 butanol – acetic acid – water system (8) was used for the second dimension. The qualitative analysis of amino acids appearing on chromatograms was accomplished by conventional identification

Received for publication on 8 April 1959.

procedures, while the densitometric measurement of spot intensity was used for quantitative analysis. In order to facilitate a more accurate measure of a greater number of amino acids, triplicate determinations were made at each of three urine volume levels (20, 40, and 60 μl.).

The amino acids thus determined were α-alanine, arginine, cysteine, glutamic acid, glutamine, glycine, histidine, lysine, methyl histidine, serine, threonine, and valine. Supplemental determinations included urine volume, creatinine (9), uric acid (10), urea (11), and sodium and potassium. Sodium and potassium determinations were accomplished with a Beckman model B spectrophotometer equipped with a flame attachment and employing an acetylene-oxygen fuel system. The urine specimens were diluted as necessary (1:12.5 or 1:50) to bring the sodium and potassium concentrations into the range of a series of six standards, and the concentrations were determined by interpolating between the two closest standards.

RESULTS

For the untimed samples, each of the amino acids (also urea and uric acid) was expressed as a ratio with creatinine, and each ratio was multiplied by 100 for convenience. For the timed samples, each of the nitrogenous constituents was expressed as a ratio with creatinine and also as hourly excretion rate.

In table I, the first line of figures for each urinary constituent presents the ratio data for untimed samples for three seasonal (total) groups. By the method of analysis of variance, seasonal variation was established for alanine, arginine, cysteine, glutamic acid, glycine, lysine, serine, urea, uric acid, and Na/K but not for glutamine, histidine, methyl histidine, threonine, or valine.

Untimed-sample data for the subjects for whom timed-sample data were obtained later are presented in each case on line 2. By comparing values in line 2 with corresponding ones in line 1, it is possible to decide whether these subgroups were representative of their respective total groups. The degree of agreement between subgroup and total group values was poorest in the summer and best in the winter, while fall data showed somewhat less agreement than was found in the winter. As was expected, the reduction in numbers of subjects made statistical assessment of seasonal variation in subgroup data difficult or even impossible. Where significant variation had been found in the total group data for alanine, cysteine, glycine, lysine, serine, and Na/K, it was no longer detectable in the subgroup data. Although the level of significance was reduced, arginine still showed significant seasonal variation when subgroup data were used. Conversely, significant variation appeared for methyl histidine in the subgroup data, although it had been absent in the data for the total groups. For urea and uric acid, the level of significance was as high for subgroup as for total group data.

Line 3 presents the subgroup data for timed samples. Comparison may be made with either line 2 or line 1, since all of these values are ratios. Timed-sample ratios for the summer subgroup for alanine, arginine, glutamine, glycine, histidine, lysine, serine, threonine, urea, and Na/K tend to be in better agreement with corresponding ones in line 1 than were line 2 values. Line 3 values for the fall and winter subgroups tend to be in good agreement with corresponding line 1 and line 2 values.

Seasonal variation could not be established statistically for line 3 data for alanine, cysteine, glutamine, glycine, histidine, lysine, methyl histidine, serine, threonine, valine, uric acid, or Na/K. This was also the case for line 4 data (hourly excretion rates) for alanine, cysteine, glycine, histidine, lysine, serine, threonine, valine, and urea, but there was significant variation with season for arginine, glutamic acid, glutamine, methyl histidine, and uric acid.

Methyl histidine is unique in that no significant variation was found for the original groups, but significant variation was found for

TABLE I

Seasonal variation in urinary amino acids

Urinary constituents	Mode of expression	Mode of collection	Summer			Fall			Winter			P*	
			n	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.		
Alanine	Ratio†	Untimed	34	0.88	0.37	19	0.72	0.36	30	0.63	0.29	< .01	
	Ratio	Untimed	7	0.55	0.24	10	0.67	0.35	11	0.62	0.26		> .05
	Ratio	Timed	7	0.80	0.59	10	0.69	0.23	11	0.78	0.38		> .05
	Rate‡	Timed	7	0.71	0.34	10	0.92	0.57	11	1.32	0.59		> .05
Arginine	Ratio	Untimed	34	1.77	1.03	19	1.29	1.03	30	2.18	1.03	< .01	
	Ratio	Untimed	7	0.94	0.89	10	1.02	1.00	11	1.91	0.57	< .05	
	Ratio	Timed	7	1.35	1.13	10	1.02	0.85	11	2.33	0.53	< .01	
	Rate	Timed	7	1.48	1.04	10	1.68	1.82	11	4.07	1.20	< .01	
Cysteine	Ratio	Untimed	34	0.32	0.32	19	0.52	0.54	30	0.15	0.10	< .01	
	Ratio	Untimed	7	0.54	0.56	9	0.36	0.21	11	0.14	0.07		> .05
	Ratio	Timed	7	0.83	0.92	9	0.36	0.25	11	0.18	0.10		> .05
	Rate	Timed	7	1.06	1.41	9	0.51	0.49	11	0.31	0.19		> .05
Glutamic acid	Ratio	Untimed	34	0.070	0.066	19	0.013	0.041	30	0.050	0.051	< .01	
	Ratio	Untimed§	7	0.008	——	10	0.000	——	11	0.020	——	——	
	Ratio	Timed	7	0.026	0.068	10	0.004	0.013	11	0.060	0.054	< .05	
	Rate	Timed	7	0.017	0.045	10	0.003	0.009	11	0.112	0.103	< .01	
Glutamine	Ratio	Untimed	34	1.28	0.47	19	1.24	0.64	30	1.24	0.49		> .05
	Ratio	Untimed	7	0.86	0.26	10	1.02	0.45	11	1.21	0.51		> .05
	Ratio	Timed	7	1.31	0.95	10	1.09	0.37	11	1.29	0.45		> .05
	Rate	Timed	7	1.18	0.51	10	1.41	0.79	11	2.21	0.80	< .05	
Glycine	Ratio	Untimed	34	2.00	0.74	19	2.74	1.26	30	1.94	1.09	< .01	
	Ratio	Untimed	7	2.07	0.98	10	2.58	1.27	11	1.85	1.12		> .05
	Ratio	Timed	7	2.33	1.49	10	2.86	1.49	11	1.86	0.84		> .05
	Rate	Timed	7	2.13	0.85	10	3.47	1.90	11	2.78	0.89		> .05
Histidine	Ratio	Untimed	34	3.00	1.37	19	2.77	1.73	30	2.97	1.57		> .05
	Ratio	Untimed	7	2.49	2.08	10	3.14	1.92	11	2.85	1.71		> .05
	Ratio	Timed	7	2.73	1.66	10	3.03	1.53	11	3.08	1.24		> .05
	Rate	Timed	7	2.74	1.70	10	3.98	2.75	11	5.33	2.37		> .05
Lysine	Ratio	Untimed	34	0.86	0.51	19	0.98	0.80	30	0.56	0.29	< .01	
	Ratio	Untimed	7	0.92	0.85	10	1.17	0.88	11	0.49	0.26		> .05
	Ratio	Timed	7	0.96	0.89	10	1.07	0.68	11	0.54	0.32		> .05
	Rate	Timed	7	0.92	0.77	10	1.32	0.70	11	0.84	0.38		> .05

Table I (continued)

Urinary constituent	Mode of expression	Mode of collection	Summer			Fall			Winter			P*	
			n	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.		
Methyl histidine	Ratio	Untimed	34	0.89	0.71	19	0.84	0.82	30	0.70	0.30		> .05
	Ratio	Untimed	7	0.10	0.21	10	0.69	0.66	11	0.58	0.27	< .01	
	Ratio	Timed	7	0.32	0.57	10	0.92	0.65	11	0.75	0.35		> .05
	Rate	Timed	7	0.30	0.51	10	0.91	0.67	11	1.34	0.71	< .05	
Serine	Ratio	Untimed	34	1.04	0.48	19	1.18	0.70	30	0.75	0.35	< .01	
	Ratio	Untimed	7	1.22	0.82	10	1.13	0.55	11	0.67	0.32		> .05
	Ratio	Timed	7	1.26	0.63	10	1.20	0.56	11	0.79	0.38		> .05
	Rate	Timed	7	1.19	0.32	10	1.40	0.63	11	1.36	0.67		> .05
Threonine	Ratio	Untimed	34	0.60	0.22	19	0.57	0.34	30	0.48	0.21		> .05
	Ratio	Untimed	7	0.50	0.28	10	0.53	0.30	11	0.47	0.23		> .05
	Ratio	Timed	7	0.65	0.47	10	0.58	0.25	11	0.47	0.17		> .05
	Rate	Timed	7	0.58	0.23	10	0.70	0.34	11	0.81	0.30		> .05
Valine	Ratio	Untimed	34	0.60	0.81	19	0.47	0.74	30	0.32	0.33		> .05
	Ratio	Untimed	7	0.17	0.22	10	0.26	0.33	11	0.19	0.17		> .05
	Ratio	Timed	7	0.32	0.54	10	0.25	0.32	11	0.33	0.24		> .05
	Rate	Timed	7	0.30	0.41	10	0.41	0.55	11	0.58	0.42		> .05
Urea	Ratio	Untimed	32	831	252	17	979	323	30	489	167	< .01	
	Ratio	Untimed	7	917	345	9	1160	233	11	508	226	< .01	
	Ratio	Timed	7	761	243	9	1102	312	11	476	123	< .01	
	Rate	Timed	7	865	465	9	1218	618	11	827	240		> .05
Uric acid	Ratio	Untimed	34	20.2	10.9	19	30.9	10.9	30	20.2	5.8	< .01	
	Ratio	Untimed	7	16.9	9.4	10	28.5	8.3	11	20.0	3.7	< .01	
	Ratio	Timed	7	29.8	41.1	10	25.9	13.0	11	23.8	5.7		> .05
	Rate	Timed	1	20.3	19.9	10	30.4	17.8	11	40.9	11.4	< .05	
Na/K		Untimed	34	2.60	1.49	18	2.37	1.26	23	1.75	0.67	< .01	
		Untimed	7	2.33	1.16	10	2.22	1.00	11	1.58	0.54		> .05
		Timed	7	2.74	1.53	10	2.83	1.22	11	3.01	2.81		> .05
Creatinine	Rate	Timed	7	106	38	10	129	68	11	174	35	< .05	
Urine volume	Rate	Timed	7	43	22	10	50	23	11	52	33		> .05

* Significance determined by analysis of variance except where there was heterogeneity, in which case it was determined by nonparametric test.

† Ratio = (mg./mg. creatinine)100.

‡ Rate = mg./hr.

§ Standard deviation for glutamic acid values in line 2 were not computed, since the majority of values were zero.

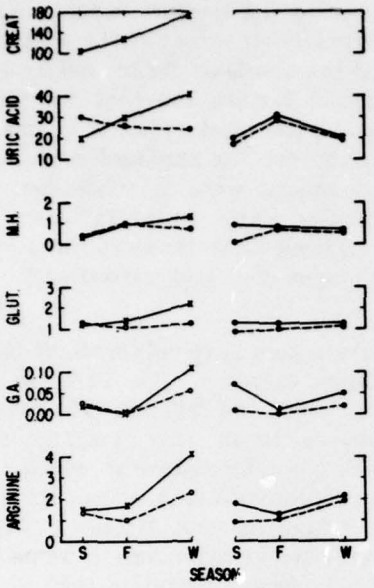

FIGURE 1

Variations with season. Data plotted in left column represent timed sample determinations: (x———x represents values expressed as milligrams per hour; o - - - o represents values expressed as ratios with creatinine). Data plotted in right column represent untimed sample determinations: (●———● represents total group ratio data; ● - - - - ● represents subgroup ratio data). G. A. – glutamic acid; Glut. = glutamine; M. H. = methyl histidine; Creat. = creatinine.

the subgroups by the use of untimed samples (with the value expressed as a ratio) and again by the use of timed samples (with the value expressed as hourly excretion rate).

The seasonal variation noted for uric acid/creatinine in the original groups was also detectable in the subgroup untimed-sample data; however, with timed-sample ratio data (obtained under standardized conditions to minimize environmental influences), seasonal effects disappeared. Still, when expressed as milligrams per hour, uric acid excretion varied significantly with season.

Seasonal variation was established for urea only when it was expressed as a ratio with creatinine; when expressed as hourly rate, the variability was not attributable to season.

A significant variation with season was found for creatinine excretion but not for urine volume.

The differences between summer and fall excretion rates for arginine, glutamic acid, glutamine, methyl histidine, uric acid, and creatinine were slight compared with those between fall and winter (fig. 1). The time interval between summer and fall determinations was less than that between fall and winter determinations. When plotted so as to provide for this difference in time intervals, an almost linear pattern appeared for creatinine, uric acid, methyl histidine, and glutamine excretion rates, but this pattern disappeared when the latter three constituents were expressed as ratios with creatinine. There was statistical significance in the rate data but not in the ratio data. Where subgroup ratio data for glutamine and methyl histidine plot in a slightly irregular manner, the total group data plot on a level line, indicating marked constancy despite seasonal factors. Such constancy was lacking in untimed-sample data for uric acid, but it should be noted that it is the fall value, not the winter value, which is out of line. While the fall value cannot be ignored, the striking agreement between summer and winter values (total group data) is evidence that uric acid excretion, like glutamine and methyl histidine, tends to remain proportional to creatinine.

Arginine and glutamic acid excretion rates followed a pattern of increase between fall and winter, and this pattern was not obliterated by the use of ratio data. Total group data conform to the same pattern found with the use of timed-sample subgroup data (ratio data); this finding suggests that the controlling mechanisms for arginine and glutamic acid are not the same as those for creatinine, uric acid, methyl histidine, and glutamine. From the fact that the fall-winter difference was of lesser magnitude when arginine and glutamic acid

were expressed as ratios with creatinine than when expressed as rates, it appears that they were controlled to some extent by the mechanism which controlled creatinine, but it was not the sole mechanism.

DISCUSSION

We are aware that untimed and short-period urine collections may give unreliable results. Contrary to older reports, recent ones indicate that creatinine excretion is not constant in individuals, and short-period collections are likely to give erroneous results (12-14). According to Miller and Blyth (13), the day-to-day variation in creatinine excretion for an individual may amount to as much as 20 percent above or below his mean (determined from five successive standardized samples). Vestergaard and Leverett (14) found, with 2-hour collections, that some individuals showed as much as 20 percent variation around their means, but this occurs only 1 time out of 10. Furthermore, they reported that only 4 out of 18 persons showed coefficients of variation greater than 10 percent, and they suggested that the immediate cause of the variation in creatinine excretion in short-period collections is variation in glomerular filtration rate.

The variations with which the above-mentioned investigators have been concerned are those which occur in persons who are studied under carefully controlled environmental conditions. Such variation may be of minor importance when an overwhelming influence such as high ambient temperature becomes the dominant one. In the present experiment, determinations which were made in February and March cannot have been greatly influenced by thermal factors in the environment, since adequate clothing was worn and indoor temperatures were kept at the comfort level. Furthermore, outdoor temperatures were such that lightweight clothing was adequate. In June, July, and August, the subjects slept in quarters which were not air-conditioned; consequently, they were exposed daily to temperatures far above the comfort level. Additionally, the majority engaged in outdoor sports. In October and November outdoor temperatures tend to remain close to the comfort range. With the creatinine excretion value for the winter group considered the one least influenced by adverse environmental factors and that for the summer group as the most affected, it was a surprise to find that the standard deviations for these two groups were very similar. There was much more scatter in the fall group, which may be evidence that these subjects were in different stages of deacclimatization.

Dietary factors have only a slight influence on creatinine excretion (12, 14). In animal studies, Treichler and Mitchell (15) found that endogenous nitrogen excretion, in general, varied with the temperature to which the animals were accustomed rather than the plane of nutrition. Radigan and Robinson (16) and Kenney (17) noted reductions in renal plasma flow and glomerular filtration rate in human subjects during acute exposure to high environmental temperature. Smith et al. (18) found that heat and exercise in combination had a greater effect on renal plasma flow and glomerular filtration rate than resulted from either factor alone; furthermore, they found that urine flow ordinarily tends to be independent of glomerular filtration. In the present results, urine volume showed no significant seasonal variation, although there was significant variation for creatinine. In dogs studied through autumn and winter months, Pitesky and Last (19) observed glomerular filtration rate depression during hot weather along with reduced tubular reabsorption capacity, and the suggestion was made that this is the result of a reduction in numbers of functioning nephrons. Czaczkes et al. (20) reported that uric acid excretion is governed by glomerular filtration rate and subsequent tubular reabsorption. Nichols et al. (21) found that the depression in uric acid excretion rate which was induced by exercise was proportional to the concomitant decrease in renal plasma flow and glomerular filtration rate.

Such facts make it seem likely that the low summer values for creatinine and uric acid are due primarily to renal changes. Since methyl

histidine and glutamine tend to remain proportional to creatinine, the mechanisms of control seem to be the same as for creatinine. Since the proportionality for glutamic acid and creatinine and for arginine and creatinine was not constant, it does not seem that the mechanisms of control for these amino acids are the same as for creatinine.

Dent (22) has expressed the opinion that the use of amino acid/creatinine ratios is permissible for healthy persons. He has found that the excretion of certain amino acids varies with blood levels (urinary elevations are due to an overflow mechanism); while for others, excretion does not vary with changes in blood levels (urinary elevations are due to a renal mechanism). Evered (23) has emphasized that (a) healthy individuals have fairly fixed patterns of amino acid excretion, and genetic factors, rather than exogenous factors, are responsible for such patterns; (b) amino acid excretion does not vary with urine volume; and (c) the renal mechanism, rather than the overflow mechanism, is responsible for the slight variations in amino acid excretion noted in apparently normal people.

According to Evered (23), the influence of diet on methyl histidine is marked, and tubular reabsorption of methyl histidine is very inefficient. In the present results, methyl histidine (total group data) was strikingly constant with season; thus, there is evidence that dietary factors were constant.

Doolan et al. (24) found that the excretion of alanine, arginine, lysine, and valine for the human is not readily elevated by increasing plasma levels, whereas the excretion of histidine, serine, glycine, and threonine increases with plasma load. With this information, the variations with season found for alanine, arginine, and lysine (total group data) must be considered to be due to the renal rather than the overflow mechanism. Since histidine and threonine (expressed either as ratios or as rates) did not vary with season, it does not seem that plasma concentrations differed with season. The variations noted for glycine and serine (total group data) apparently are due to renal mechanisms.

Some of the results for the fall group are difficult to interpret. This is because the values were not intermediate with respect to summer and winter values. Arginine, cysteine, glutamic acid, glycine, lysine, serine, urea, and uric acid (total group data) show such a peculiarity. Stein et al. (25) have shown that deacclimatization for heat is a slow process in the human, requiring weeks or months. Possibly the greater degree of scatter and the peculiar displacements noted in the fall are indicative of deacclimatization changes. The relatively high uric acid/creatinine value in the fall (total group data) is an indication of a disturbed state. The relatively high value and wide variation found for urea in the fall also suggest a disturbed state.

Compared with values reported by Ralli et al. (26) for healthy young males, the values for creatinine excretion (mg./hr.) obtained here are high; however, there is excellent agreement between uric acid excretion rate in the fall group in the present study and the group studied by Ralli et al. Compared with values obtained in patients who were studied in a hot-dry climate by Mefferd et al. (1), the summer urea value (rate data) obtained here is slightly high, the creatinine and uric acid values (rate data) are very high, while the Na/K values (timed-sample data) are in close agreement.

The authors are grateful for the help of Richard C. McNee, Department of Biometrics, who performed the statistical analyses.

REFERENCES

1. Mefferd, R. B., Jr., E. H. LaBrosse, A. M. Gawienowski, and R. J. Williams. Influence of chlorpromazine on certain biochemical variables of chronic male schizophrenics. J. Nerv. & Ment. Dis. 127:167-179 (1958).

2. Mefferd, R. B., Jr., H. B. Hale, and H. H. Martens. Nitrogen and electrolyte excretion of rats chronically exposed to adverse environments. Am. J. Physiol. 192:209-213 (1958).

3. Hale, H. B., and R. B. Mefferd, Jr. Factorial study of environmentally-induced metabolic changes in rats. Am. J. Physiol. 194:469-475 (1958).

4. Mefferd, R. B., Jr., and H. B. Hale. Effects of abrupt temperature changes on excretion characteristics of rats acclimated to cold, neutral, or hot environments. Am. J. Physiol. 195: 726-734 (1958).

5. Consden, R., A. H. Gordon, and A. J. P. Martin. The identification of lower peptides in complex mixtures. Biochem. J. 41:590-596 (1947).

6. Awapara, J. Application of paper chromatography to the estimation of free amino acids in tissues. Arch. Biochem. 19:172-173 (1948).

7. Levy, A. L., and D. Chung. Two-dimensional chromatography of amino acids on buffered papers. Analyt. Chem. 25:396-399 (1953).

8. Partridge, S. M. Filter-paper partition chromatography of sugars. 1. General description and application to qualitative analysis of sugars in apple juice, egg white, and foetal blood of sheep. Biochem. J. 42:238-248 (1948).

9. Folin, O., and H. Wu. A system of blood analysis. J. Biol. Chem. 38:81-110 (1919).

10. Benedict, S. R., and E. Franke. A method for the direct determination of uric acid in urine. J. Biol. Chem. 52:387-391 (1922).

11. Ormsby, A. A. A direct colorimetric method for the determination of urea in blood and urine. J. Biol. Chem. 146:595-604 (1942).

12. Best, W. R. Physiologic factors in urinary creatinine excretion. Medical Nutrition Laboratory, U. S. Army, Report 118, October 1953.

13. Miller, A. T., Jr., and C. S. Blyth. Estimation of lean body mass and body fat from basal oxygen consumption and creatinine excretion. J. Appl. Physiol. 5:73-78 (1953).

14. Vestergaard, P., and R. Leverett. Constancy of urinary creatinine excretion. J. Lab. & Clin. Med. 51:211-218 (1958).

15. Treichler, R., and H. H. Mitchell. The influence of plane of nutrition and of environmental temperature on the relationship between basal metabolism and endogenous nitrogen metabolism subsequently determined. J. Nutrition 22:333-343 (1941).

16. Radigan, L. R., and S. Robinson. Effects of environmental heat stress and exercise on renal blood flow and filtration rate. J. Appl. Physiol. 2:185-191 (1949).

17. Kenney, R. A. The effect of hot, humid environments on the renal function of West Africans. J. Physiol. 118:25-26 P (1952).

18. Smith, J. A., S. Robinson, and M. Pearcy. Renal responses to exercise, heat, and dehydration. J. Appl. Physiol. 4:659-665 (1952).

19. Pitesky, I., and J. H. Last. Effects of seasonal heat stress on glomerular and tubular functions in the dog. Am. J. Physiol. 164:497-501 (1951).

20. Czaczkes, W. J., T. D. Ullman, and E. Sadowski. Plasma uric acid levels, uric acid excretion, and response to probenecid in toxemia of pregnancy. J. Lab. & Clin. Med. 51:224-229 (1958).

21. Nichols, J., A. T. Miller, Jr. and E. P. Hiatt. Influence of muscular exercise on uric acid excretion in man. J. Appl. Physiol. 3:501-507 (1951).

22. Dent, C. E. The renal amino-acidurias. Exper. Med. & Surg. 12:229-232 (1954).

23. Evered, D. F. The excretion of amino acids by the human. A quantitative study with ion-exchange chromatography. Biochem. J. 62:416-427 (1956).

24. Doolan, P. D., H. A. Harper, M. E. Hutchin, and W. W. Shreeve. Renal clearance of 18 individual amino acids in human subjects. J. Clin. Invest. 34:1247-1255 (1955).

25. Stein, H. J., J. W. Eliot, and R. A. Bader. Physiological reactions to cold and their effects on the retention of acclimatization to heat. J. Appl. Physiol. 1:575-585 (1949).

26. Ralli, E. P., W. J. Kuhl, Jr., H. Gershberg, E. M. Beck, E. R. Street, and B. Laken. Effects of vitamin supplementation of the diet on reaction to short-term cold stress in normal young male adults. Metabolism 5:170-196 (1956).